provide formal drawings upon notification of allowable subject matter. The specification has been amended to include material incorporated by reference. The relevant material is inserted by the above amendment to the specification. The undersigned verifies that the amendatory material consists of the material incorporated by reference in the referencing application. No new matter has been added.

Objection to Claim 11 Under 37 CFR § 1.75(c)

The Examiner has objected to claim 11 as allegedly being in improper dependent form. Claims 10 and 11 have been amended to refer to claims in the alternative ("claims 1, 2, 3, 4, or 5"). In light of these amendments, applicants request that the Examiner examine claim 11 on the merits.

Rejection of Claims 15 and 16 Under
35 U.S.C. §112, first paragraph

The Examiner has rejected claims 15 and 16 as directed to "subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected to make and/or use the invention." Applicants respectfully traverse this rejection.

At page 3 of the Office Action the Examiner states that

> [w]ith respect to claims 15 and 16, the application is silent with respect to the role of Helios in any disorder or any examples that point to any specific function of Helios. Sequence homology demonstrates that Helios is a member of the T cell-restricted Ikaros family and experiments show that Helios can interact with other family members. It has been demonstrated that the absence of one of the family members, Ikaros, can lead to development disorders. However, this is complicated by the fact that expression of some mutant forms of Ikaros produce effects different than that of the complete absence, suggesting complicated mechanisms of action or control of Ikaros.

The observation to which the Examiner refers is compelling evidence of the importance of Ikaros-related genes in development, specifically in the early stages of hematopoietic cell development. As described in the Kelley *et al.* reference cited by the Examiner ((1998) Curr. Biol. 8:508-515), two types of mice possessing defects in the Ikaros gene have been generated:

Ikaros null mice and mice expressing a dominant negative Ikaros mutation. Interestingly, mice expressing the dominant negative Ikaros mutation have a more severe phenotype than mice lacking Ikaros altogether. Specifically, the Ikaros null mice display defects in lymphoid development, whereas the dominant negative Ikaros mice show more severe lymphoid defects as well as defects in hematopoietic stem cells. The dominant negative form of Ikaros lacks a DNA binding domain but possesses a dimerization domain. Thus, the presence of the Ikaros dimerization domain is thought to modulate the function of an Ikaros-like factor that dimerizes with Ikaros, thereby disrupting the activity of the Ikaros-like factor and causing a disturbance in the hematopoietic stem cell compartment. As described by Kelley *et al.*, the effect of the Ikaros dominant negative mutant suggests the existence of an additional factor that can: (i) dimerize with Ikaros; and (ii) partially complement its function. Thus, the experiments to which the Examiner refers, rather than complicating the understanding of Ikaros, suggest an essential role in hematopoietic stem cell development for an Ikaros-related protein that dimerizes with Ikaros.

Applicants have cloned a new member of the Ikaros gene family, designated Helios. Applicants have disclosed Helios nucleic acids and polypeptides and have provided significant insight into the *in vivo* function of Helios. For example, applicants have shown that Helios shares significant sequence similarity with other members of the Ikaros gene family, Ikaros and Ailos. Applicants have demonstrated that Helios forms dimers with Ikaros and Ailos. Applicants have shown that Helios is expressed in a developmentally regulated fashion, e.g., predominant expression by hematopoietic stem cell populations and diminished expression by differentiated hematopoietic cells (*see, e.g.*, page 64, line 9 to page 67, line 22). Taken together, these constitute compelling evidence that Helios, a member of the Ikaros "master switch" family of genes, plays an important role in hematopoietic stem cell development (*see, e.g.*, page 67, lines 11-13 stating that "this profile of Helios expression suggests that transcriptional complexes including Ikaros and Helios will predominate in the early stages of hematopoiesis"). Applicants' discovery demonstrates that misexpression of the Helios gene is likely to result in developmental defects of hematopoietic cells, particularly hematopoietic stem cells.

In light of applicants' detailed characterization of the Helios cDNA and the Helios protein, the present application provides sufficient guidance to enable a skilled artisan to treat disorders characterized by a misexpression of Helios (claim 15) and to determine if a subject is at

risk for a disorder related to misexpression of the Helios gene (claim16). With respect to method of treating an animal of claim 15, the application provides detailed instruction on the administration to an animal of each of: a therapeutically active amount of an Helios polypeptide; a cell selected for the expression of a product of an Helios gene; and a nucleic acid encoding an Helios peptide. With respect to the method of claim 16, the application gives detailed disclosure of the sequence of the Helios cDNA and how to use this disclosed sequence to detect expression or structure of the Helios gene. As described above, applicants have characterized functional properties of Helios, expression patterns of Helios, and the likely role of Helios in hematopoietic cell development.

At pages 36-40 of the specification, applicants provide a detailed description of methods and vectors that can be used to introduce Helios nucleic acid molecules into a subject. In addition, applicants have provided numerous methods of enhancing plasmid DNA delivery for gene therapy. Moreover, at the time of filing of the instant application, there were several developments in gene therapy delivery systems and gene therapy expression. For example, Zufferey et al. (1997) *Nature Biotechnol.* 15:871-875 (submitted herewith as Exhibit B), report successful *in vivo* delivery of a reporter gene using retroviral vectors derived from lentoviruses such as HIV-1. Zufferey et al. state that such vectors are "promising tools for human gene therapy because they mediate *in vivo* delivery and long-term expression of transgenes in nondividing tissues." Thus, at the time of invention, methods were known for enhancing delivery and expression of genes for gene therapy.

Thus, based on the knowledge in the art and the teachings in the present application, one of ordinary skill in the art could practice the claimed invention without undue experimentation.

The Examiner has cited Verma *et al.* (1997) *Nature* 389:239-242 and Anderson (1998) *Nature* 392:25-30, to assert that "even if the function of Helios was known, or what disease is associated with a particular expression or form of Helios, the *in vivo* or *ex vivo* gene therapy methods to deliver the DNA or corresponding peptide would involve undue experimentation" (page 4 of Office Action). Specifically, the Examiner cites Anderson as disclosing that

> [t]hus far, the problem has been an inability to deliver genes efficiently and to obtain expression [and that] there is still no conclusive evidence that a gene-therapy protocol has been successful in the treatment of a human disease [and

> that] [s]everal major deficiencies still exist including poor delivery systems, both viral and non-viral, and poor gene expression after genes are delivered.

Applicants submit that the state of gene therapy is not as dismal as characterized by the Examiner. As evidence of this, applicants direct the Examiner's attention to a publication in Science (Crystal, R (1995) *Science* 270:404-410; a copy of which is submitted herein as Exhibit C) in which the state of human gene therapy is reviewed. The author concludes that human gene transfer is feasible and states as follows (at page 405):

> probably the most remarkable conclusion drawn from the human trials is that human gene transfer is indeed feasible. Although gene transfer has not been demonstrated in all recipients, most studies have shown that genes can be transferred to humans whether the strategy is *ex vivo* or *in vivo*, and that all vector types function as intended. Taken together, the evidence is overwhelming, with successful human gene transfer having been demonstrated in 28 ex vivo and 10 in vivo studies. (emphasis added)

Furthermore, at the time of the filing of the instant application, there was evidence that gene therapy was successful. For example, the Journal of the American Medical Association (Randall, T (1993) *JAMA* 269:837-838; a copy of which is submitted herewith as Exhibit D) reported the partial success of a gene therapy treatment for inherited hypercholesterolemia. A modified retrovirus containing the LDL receptor gene was used to infect hepatocytes from the patient. The procedure was estimated to correct the defect in approximately 3-5% of the patient's cells, the physician in charge of the case stated that "even a small change is significant." In addition, the U. S. Patent and Trademark Office has recognized the utility of gene therapy by issuing claims to gene therapy, see for example, U.S. patent 5,672,344, directed to viral-mediated gene transfer (submitted herewith as Exhibit E).

Therefore, applicants submit that the specification as filed is enabling for the invention and respectfully requested that the Examiner withdraw the rejection of claims 15 and 16.

Rejection of Claims 3-5, 15, and 16 Under
35 U.S.C. §112, second paragraph

The Examiner has rejected claims 3-5 as allegedly vague and unclear in their recitation of "Helios polypeptide." Each of these claims has been amended to refer to a specific nucleotide

sequence (SEQ ID NO:1, 3, or 5) or amino acid sequence (SEQ ID NO:2, 4, or 6) in place of the reference to a "Helios polypeptide." Withdrawal of the rejections is requested.

The Examiner has rejected claim 3 as allegedly vague and indefinite in its recitation of "under high stringency conditions." Page 56, lines 28-29 of the application refers to the definition of "high stringency conditions." The specification has been amended to include the hybridization conditions that were originally incorporated by reference. These amendments obviate the Examiner's rejection of the claims. Therefore, applicants respectfully request that the Examiner withdraw this rejection.

The Examiner has rejected claims 15 and 16 as allegedly vague and indefinite "because it is not clear what disorder is being treated or diagnosed. Neither the claim nor the specification describe Helios related disorders." Applicants respectfully traverse this rejection. The application discloses the sequence of the Helios cDNA and how to use this sequence to examine expression of Helios or the structure of the Helios gene. As described above with respect to the § 112, paragraph 1 rejection, the application further describes hematopoietic cell expression of Helios and its likely function in hematopoietic cell development. Thus, the application provides ample disclosure of Helios-related functions and Helios-related disorders. Applicants request that the rejections be withdrawn.

Rejection of Claims 1-5 Under
35 U.S.C. §102(a)

The Examiner has rejected claims 1-5 as allegedly anticipated by Hahm *et al.* (GenBank™ Accession Number AF044257). Hahm *et al.* was published on February 4, 1998, less than a year before the February 27, 1998 priority date to which these claims are entitled. The enclosed Declaration under 37 CFR § 1.131 establishes a date of invention in the U.S. prior to February 4, 1998, before the Hahm et al. publication date. The provisional application to which the present application claims priority was filed on February 27, 1998, thus Applicants acted with diligence. Thus, Hahm *et al.* is not prior art against these claims under § 102(a). Withdrawal of the rejections is respectfully requested.

Rejection of Claims 1-5, 10, 11, and 13 Under

35 U.S.C. §103(a)

The Examiner has rejected claims 1-5, 10, 11, and 13 as allegedly obvious over Hahm *et al.* in view of Molnar *et al.* (1994) *Mol. Cell. Biol.* 14:8292-8303. As detailed above with respect to the § 102(a) rejection, Hahm *et al.* was published on February 4, 1998, less than a year before the February 27, 1998 priority date to which these claims are entitled. Because Hahm *et al.* is not prior art against these claims, applicants request withdrawal of the obviousness rejections.

CONCLUSION

Applicants submit that all of the claims are now in condition for allowance, which action is requested. Filed herewith is a Petition for Automatic Extension with the required fee. Please apply any other charges or credits to Deposit Account No. 06-1050, referencing attorney docket number 10287-043001.

Respectfully submitted,

Date: 9/29/00

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